CHROMOSOME LOCATION AND LINKAGE STUDIES INVOLVING THE *Pm3* LOCUS FOR POWDERY MILDEW RESISTANCE IN WHEAT

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(Plates xvi–xvii)

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Synopsis

The chromosome location of the incompletely dominant gene conditioning resistance to powdery mildew in Asosan wheat was confirmed as 1A by nullisomic F_2 analysis. This gene, formerly designated Ml_a, is now known to be an allele at the *Pm3* locus and is redesignated *Pm3a* following the recommendations for gene nomenclature in wheat. Repulsion phase linkage studies, using F_2 genotypic classification verified from progeny tests, indicated a crossover value of $4{\cdot}81 \pm 0{\cdot}52$ per cent between *Pm3a* and the gene *Hg* conditioning pubescent glumes.

Introduction

The loci for certain of the genes conferring resistance to powdery mildew, *Erysiphe graminis* DC. f. sp. *tritici* Em. Marchal, in common or bread wheat, *Triticum aestivum* L. ssp. *vulgare* (Vill.) have been located on specific chromosomes by aneuploid analyses (Sears and Rodenhiser, in Sears (1954) ; Nyquist, 1957; Briggle and Sears, 1966; Law and Wolfe, 1966; McIntosh, Luig and Baker, 1967). In other instances linkage values have been determined between genes for mildew resistance and previously localised factors which condition other characters (*e.g.*, Briggle and Sears, 1966). Such studies on gene location and linkage intensity have contributed to the rapid expansion of the wheat genetic map in recent years. The studies to be reported are concerned with the chromosome location of a gene conferring mildew resistance in the variety Asosan and with the determination of the linkage value between this gene and that conditioning pubescent glumes.

Literature Review

Pugsley (1961) identified a dominant gene Ml_a for seedling resistance to powdery mildew in the wheat variety Asosan. Briggle (1966) assigned permanent numerals to three loci conditioning mildew resistance, giving the symbol *Pm3* to the locus at which Ml_a is located. Briggle and Sears (1966) reported the presence of a multiple allelic series at this locus in various wheat varieties. Alternatively they proposed that close linkage could be implicated. On the basis of allelism they suggested that as many as five alleles were involved. One group of varieties included Asosan, in which the gene for resistance was effective from the first-leaf stage to maturity. A second group included the variety Indian 1A, in which resistance was not expressed until the three to four-leaf stage. In the studies by Briggle and Sears tests for mildew reactions on the progenies of disomic F_2 plants from

crosses between the Chinese Spring monosomic series and Indian identified no chromosome unequivocally as the carrier of *Pm3*. However, tests of Indian substitution line 1A, in which chromosomes 1A of Indian were substituted for their homologues in Chinese Spring by means of a series of six backcrosses to Chinese Spring monosomic 1A, showed clearly that this chromosome carried *Pm3*.

A single gene difference between pubescent and glabrous glumes has been indicated in most instances (see Ausemus *et al.* (1946)). Sears (1953) by nullisomic analysis placed the single dominant gene for pubescent glume *Hg* (Tsunewaki, 1966) in the variety Indian on chromosome 1A.

From backcross data Briggle and Sears (1966) found strong linkage in coupling between *Hg* and *Pm3*, the crossover value being 0·82 map units.

Materials and Methods

A backcross mildew-resistant derivative Asosan × Federation³ W2583* was used in the investigations. It carries the mildew-resistance gene Ml_a from Asosan, possesses glabrous glumes and is distinguished morphologically from Federation in having grains with red pericarp.

The chromosome location of Ml_a was determined by crossing Asosan × Federation³ as the pollen parent with the series of twenty-one Chinese Spring monosomics, which are mildew susceptible. Monosomic F_1 plants were distinguished from disomic sibs by meiotic examinations of pollen mother cells, stained with acetocarmine, from anthers in spikes fixed in Farmer's fixative. The segregation ratios for reaction types on the primary seedling leaves were studied in the progenies of monosomic F_1 plants in each cross.

The mildew susceptible variety Yalta W1373, with pubescent glumes due to the gene *Hg*, was crossed with Asosan × Federation³ in order to estimate the recombination value between Ml_a and *Hg*. The progenies of five F_1 plants were studied, about half the spikes on each plant being bagged to prevent possible outcrossing. Since on previous evidence recombination between the genes under study was rare, outcrossing could affect estimates of the recombination value. Seed on bagged spikes of each hybrid plant was threshed and bulked, but kept separated from seed produced by open pollination. Each plant progeny was analysed separately. F_2 seedlings were classified for mildew reaction type on the primary leaf, sprayed with an appropriate fungicide to control further mildew development, transplanted to the field and grown to maturity. The phenotypic classification for glume pubescence was made macroscopically. In doubtful cases a binocular microscope was used for final classification. Segregates with pubescent glumes were classified into two classes, one fully pubescent and the other intermediate in degree of development and in length of the trichomes on the glumes.

The mildew reactions of progenies from F_2 plants classified for mildew reaction type and degree of glume pubescence were determined. On the basis of these tests residue seed from apparent F_2 recombinants between Ml_a and *Hg* was space-planted in the field and F_3 lines classified for pubescent versus glabrous glumes at maturity.

A strain of wheat powdery mildew designated S.U.1 (McIntosh and Baker, 1966) was used in the investigations. Mildew reaction types were scored according to the scheme described by Newton and Cherewick (1947).

* Refers to Sydney University Wheat Accession Register.

Experimental Results

Chromosome location of Pm3 *locus*

Asosan × Federation[3] was virtually immune to mildew, exhibiting "0;" reaction types on the primary seedling leaf, in contrast to the susceptible "3+" reaction type pustules shown by the Chinese Spring monosomics. Asosan × Federation[3] showed some mildew on the coleoptile, but this was ignored in the investigations. Hybrid plants exhibited slightly higher ("1") reaction types, indicating incomplete dominance of the resistant reaction type. Segregation ratios for mildew reaction type in populations from monosomic F_1 plants from crosses involving various Chinese Spring monosomics are presented in Table 1. Some resistant seedlings showed reaction types similar to Asosan × Federation[3], but the majority, presumably heterozygous in genotype, exhibited "1" types similar to the F_1 plants. Populations from

Table 1

Segregation for seedling mildew reaction type in progenies of monosomic F_1 plants from crosses between the various Chinese Spring monosomic lines and Asosan × Federation[3]

Chromosome involved	Reaction types		Total	$\chi^2_{(3:1)}$	P value
	"0;, 11+" (resistant)	"3+" (susceptible)			
1A	224	15	239	44·69	0·001
1B	119	40	159	0·002	0·99–0·95
1D	51	15	66	0·18	0·95–0·50
2A (II)	43	15	78	0·02	0·95–0·50
2B (XIII)	60	16	76	0·63	0·50–0·20
2D	62	18	80	0·27	0·95–0·50
3A	52	19	71	0·12	0·95–0·50
3B	48	16	64	0·00	1·00
3D	44	19	63	0·89	0·50–0·20
4A	78	27	105	0·03	0·95–0·50
4B	43	20	63	1·53	0·50–0·20
4D	54	20	74	0·16	0·95–0·50
5A	51	21	72	0·67	0·50–0·20
5B	56	16	72	0·30	0·95–0·50
5D	17	8	25	0·65	0·50–0·20
6A	69	16	85	1·73	0·20–0·10
6B	33	7	40	1·20	0·50–0·20
6D	65	14	79	2·23	0·20–0·10
7A	49	12	61	0·01	0·95–0·50
7B	38	18	56	1·52	0·50–0·20
7D	44	18	62	0·54	0·50–0·20
Total (excluding 1A)	1,076	355	1,431	0·03	0·95–0·50

20 of the 21 monosomics gave segregation ratios conforming with expectation for a single incompletely dominant factor pair in Asosan. For the cross involving monosome 1A (XIV) a highly significant deviation ($P < 0{\cdot}001$) was shown on this hypothesis. The deficiency in the number of susceptible segregates in the cross involving only 1A implies that the gene conditioning mildew resistance in Asosan is located on this chromosome.

Three seedlings chimaeric for reaction types ("1–" and "3+") closely approaching the parental types were observed among the progeny of the monosomic 1A F_1 plant (Pl. xvi). In all cases the longitudinal division line between the resistant and susceptible sectors was at, or very close to, the leaf midrib. Presumably chimaerism resulted from loss in an early embryonic division of the Asosan 1A chromosome in a seedling monosomic or monotelosomic for this chromosome. Chimaeric seedlings were grouped with the resistant class on the basis of this assumed origin.

Linkage intensity between Pm3 *and* Hg

The genotypes of Asosan × Federation[3] and Yalta can be designated *Pm3Pm3 hghg* and *pm3pm3 HgHg* respectively and linkage was studied therefore in the repulsion phase. In mildew resistant F_2 segregates two distinct groups of seedlings were observed. One group exhibited "0;" reaction types similar to the resistant parent and the other "11+" reaction types. Tests in F_3 confirmed the latter class as the heterozygous genotype. Glume pubescence was also incompletely dominant. The intermediate phenotype in the F_2 group is shown in Plate XVII together with the fully pubescent and glabrous glumed phenotypes. Behaviour of F_3 again confirmed that the intermediate class was heterozygous. Behaviour of F_3 lines for mildew reaction verified generally the accuracy of F_2 genotypic classification for mildew reaction type and indicated few misclassification errors.

An inspection of F_2 data, in which the genotypic classification for mildew reaction type was verified or corrected from progeny tests, indicated certain recombinant classes resulting from crossing over between *Pm3* and *Hg* in repulsion in F_1 gametogenesis. Individuals in such classes were checked by F_3 tests for the correctness or otherwise of F_2 genotypic classification at the *Hg* locus. Progeny tests for this purpose were of homozygous resistant plants which were pubescent in F_2, of plants heterozygous for mildew reaction type classified as homozygous dominant or homozygous recessive at the *Hg* locus, and of homozygous susceptible plants classified as heterozygous at the *Hg* locus. Lines in which plants were scored for pubescent versus glabrous glumes at maturity confirmed genetic recombination and revealed accurate classification of *Hghg* and *hghg* F_2 genotypes. In 3 cases out of 36, plants classified as homozygous pubescent in F_2 were found to be heterozygous on the basis of F_3 tests. No significant differences in segregation were found in the populations derived from bagged versus open-pollinated F_1 spikes and the data were pooled for analysis. The F_2 genotypic totals for mildew reaction type, confirmed or amended in a few instances on the basis of progeny tests, were 216 homozygous resistant, 432 heterozygous and 194 homozygous susceptible. However, 44 seedlings classified for reaction type to mildew failed to survive transplantation and produced neither adult plants for pubescent glume classification nor seed for progeny testing. These were not distributed at random in the three mildew reaction categories, 9 being from the homozygous resistant, 14 from the heterozygous and 21 from the homozygous susceptible classes. Despite spraying for mildew control, survival was strongly biassed against susceptible seedlings. The final figures were adjusted therefore in each mildew reaction genotype. F_2 plants which died were included to remove the possible effect of differential survival on linkage estimation. The distribution within glume pubescence genotypes of the plants which failed to survive was based on that shown for surviving plants in each category. The adjusted numbers in the different genotypes for mildew reaction and pubescence genotypes are shown in Table 2.

From maximum likelihood equations, recombination between *Pm3* and *Hg* was calculated to be $4{\cdot}81 \pm 0{\cdot}52$ per cent.

Discussion and Conclusions

The current investigations using nullisomic analysis demonstrated that chromosome 1A carried the gene Ml_a for mildew resistance in Asosan. This confirmed the findings of Briggle and Sears (1966) who used the chromosome substitution technique to place an allele in Indian on this chromosome. Briggle (1966) designated the locus at which Ml_a is situated as *Pm3* and

proposed that alleles at a locus be indicated by lower case letters. On the basis of this recommendation we propose that the gene in Asosan previously referred to by Pugsley (1961) as Ml_a be designated *Pm3a*. This symbolism seems logical and orderly, especially as this gene exhibits the lowest reaction type on the primary seedling leaf of alleles at this locus.

The postulated origin of chimaeras for mildew reaction in three F_2 seedlings in the progeny of a monosomic 1A F_1 plant provided additional evidence that chromosome 1A carried the *Pm3* locus. In disomic heterozygotes it would be necessary to invoke an unusually high rate of mitotic instability to explain their occurrence. It is highly probable that such seedlings were initially monosomic for chromosome 1A carrying the *Pm3a* allele and hence

Table 2

Numbers of plants in various F_2 genotypes from five hybrids between Asosan×Federation[3] (Pm3aPm3a hghg) *and Yalta* (pm3apm3a HgHg)*

Genotype	Number of plants
Pm3aPm3a HgHg	1·04
Pm3aPm3a Hghg	18·75
Pm3aPm3a hghg	205·21
Pm3apm3a HgHg	14·45
Pm3apm3a Hghg	403·67
Pm3apm3a hghg	27·88
pm3apm3a HgHg	195·05
pm3apm3a Hghg	19·95
pm3apm3a hghg	0·00
Total	886·00

* Adjusted to include 9 mildew-resistant (*Pm3aPm3a*) plants, 14 plants heterozygous for mildew reaction type (*Pm3apm3a*) and 21 mildew-susceptible (*pm3apm3a*) plants which failed to survive after transplantation.

χ^2_2 (1 *Pm3aPm3a* : 2 *Pm3apm3a* : 1 *pm3apm3a*) = 0·26 ; P = 0·95–0·50.

χ^2_2 (1 *HgHg* : 2 *Hghg* : 1 *hghg*) = 1·15 ; P = 0·95–0·50.

hemizygous for this gene. A high rate of mitotic instability is more characteristic of monotelocentric than normal chromosomes in *Agropyron* addition lines to wheat (Baker, unpublished) ; Steinitz-Sears (1966) reported also that telocentrics for the short arm of monosome 3B in wheat were unstable somatically. This suggests that the chimaeric seedlings may have been produced, in fact, from individuals monotelocentric for the arm of 1A carrying the *Pm3a* gene. Assuming the constitution of the chimaeric sectors is as postulated, a comparison of the reaction types in the resistant sector with those exhibited by Asosan × Federation[3] (Pl. xvi) indicates that *Pm3a* is slightly less effective in the hemizygous than the homozygous state. However, the reaction types appeared lower than in the heterozygous state in identical backgrounds under the same environmental conditions.

In crosses both with Chinese Spring monosomics and Yalta, *Pm3a* was incompletely dominant. The heterozygous genotypes in crosses with Yalta showed somewhat higher ("11+") reaction types than with Chinese Spring ("1"). This may have been due to the higher temperatures prevailing when the Yalta crosses were tested.

The estimate of linkage intensity between *Pm3* and *Hg* in coupling by Briggle and Sears (1966) from testcross data, using the variety Chancellor with the double recessive genotype as the male parent, indicated close linkage with a crossover value of 0·82 per cent. The value of 4·81 ± 0·52 per cent in the current investigations from F_2 studies with linkage in repulsion is significantly higher. In maize there is evidence that crossing over for many of the chromosomes is considerably higher in male than female gametogenesis (Rhoades, 1941; Burnham, 1949). Briggle and Sears estimate restricted crossing over to female gametogenesis whereas in the current studies crossing over occurred in both sexes. It is not known how widespread the phenomenon of higher crossing over in the pollen is in higher plants. Ramage (1960), in fact, found crossing over higher in the female in barley. In any case estimates of linkage frequently vary in different investigations. In barley, for example, Woodward (1957) and Wells (1958) obtained recombination percentages of 26·5 and 18·0 respectively between the loci for rough versus smooth awn (*Rr*) and long versus short-haired rachilla (*Ss*).

It is of interest that Briggle (1966) described a group of five mildew resistant varieties, all with pubescent glumes, in which resistance was not expressed until the three to five-leaf stage and in which three different alleles appeared to be involved at the *Pm3* locus. Selections have been made in appropriate F_3 lines in the current investigations to isolate *Pm3aPm3a HgHg* genotypes. These together with the progeny of the single *Pm3aPm3a HgHg* recombinant F_2 individual classified in the studies will furnish a useful genetic stock carrying two dominant closely linked markers which are readily classifiable genotypically and in which the mildew reaction classification can be made at the primary leaf stage.

Briggle and Sears (1966) from the phenotype of a plant monotelosomic for the long arm of an Indian chromosome 1A in a Chinese Spring background and an analysis of its progeny concluded that the *Pm3* and *Hg* loci were both on the short arm of this chromosome. The distances of each gene from the centromere can be determined by using a telocentric for the short arm of 1A in mapping but as yet this aneuploid stock is not available. In view of the close proximity of the two loci it will be of interest to determine if the technique is sufficiently precise to place their order with respect to the centromere should the genes be situated at some distance from it. Should the loci be close to the centromere the difference between the recombination value obtained in the current investigations and that published by Briggle and Sears (1966) may be due, in part at least, to differences in frequencies of crossing over in male and female gametogenesis since Rhoades (1941) found differences more accentuated in regions near the centromeres in maize.

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References

Ausemus, E. R., Harrington, J. B., Reitz, L. P., and Worzella, W. W., 1964.—A summary of genetic studies in hexaploid and tetraploid wheats. *J. Amer. Soc. Agron.*, 38: 1082-1099.

Briggle, L. W., 1966.—Three loci in wheat involving resistance to *Erysiphe graminis* f. sp. *tritici*. *Crop Sci.*, 6: 461-465.

————, and Sears, E. R., 1966.—Linkage of resistance to *Erysiphe graminis* f. sp. *tritici* (*Pm3*) and hairy glume (*Hg*) on chromosome 1A of wheat. *Crop Sci.*, 6: 559-561.

BURNHAM, C. R., 1949.—Crossing over differences in micro- and megasporogenesis in corn. Agron. Absts. Amer. Soc. Agron., p. 2.

LAW, C. N., and WOLFE, M. S., 1966.—Location of genetic factors for mildew resistance and ear emergence time on chromosome 7B of wheat. *Can. J. Genet. Cytol.*, 8: 462-470.

MCINTOSH, R. A., and BAKER, E. P., 1966.—Differential reactions to three strains of wheat powdery mildew (*Erysiphe graminis* var. *tritici*). *Aust. J. biol. Sci.*, 19: 767-773.

————, LUIG, N. H., and BAKER, E. P., 1967.—Genetic and cytogenetic studies of stem rust, leaf rust, and powdery mildew resistances in Hope and related wheat cultivars. *Aust. J. biol. Sci.*, 20: 1181-1192.

NEWTON, MARGARET, and CHEREWICK, W. J., 1947.—*Erysiphe graminis* in Canada. *Can. J. Res. C.*, 25: 73-93.

NYQUIST, W. E., 1957.—Monosomic analysis of stem rust resistance in a common wheat strain derived from *Triticum timopheevi*. *Agron. J.*, 49: 222-223.

PUGSLEY, A. T., 1961.—Additional resistance in *Triticum vulgare* to *Erysiphe graminis tritici*. *Aust. J. biol. Sci.*, 14: 70-75.

RAMAGE, R. T., 1960.—Differential crossing over in male and female gametes. Barley Newsletter, 3: 34.

RHOADES, M. M., 1941.—Differential rates of crossing over in male and female gametes of maize. *J. Amer. Soc. Agron.*, 33: 603-615.

SEARS, E. R., 1953.—Nullisomic analysis in common wheat. *Amer. Naturalist*, 87: 245-252.

————, 1954.—The aneuploids of common wheat. Missouri Agr. Expt. Sta. Res. Bull. 572. 59 pp.

TSUNEWAKI, K., 1966.—Comparative gene analysis of common wheat and its ancestral species. III. Glume hairiness. *Genetics*, 53: 303-311.

WELLS, S. A., 1958.—Inheritance of reaction to *Ustilago hordei* (Pers.) Lagerh. in cultivated barley. *Can. J. Plant Sci.*, 38: 45-60.

WOODWARD, R. W., 1957.—Linkages in barley. *Agron. J.*, 49: 28-32.

EXPLANATION OF PLATE XVI

Portions of leaves of F_2 segregates from monosomic F_1 plants in cross Chinese Spring monosomic 1A × (Asosan × Federation³) showing reaction types to powdery mildew. Left—resistant segregate. Middle—chimaeric segregate. Resistant (left) and susceptible (right) sectors. Right—susceptible segregate. (× 8.)

EXPLANATION OF PLATE XVII

F_2 segregation for glume pubescence in cross (Asosan × Federation³) × Yalta. Left—homozygous pubescent segregate. Middle—heterozygous pubescent segregate. Right—glabrous segregate. (× 3·75.)